REMARKS

1. By entering this Amendment, claims 1, 19-20, 22-23, 25-26, 28, 30-31, 33, 35, 37-38, 40, 43-45, and 48 will be pending in the application, with claims 19, 25, 28, 35, and 40 withdrawn from consideration.

2. The Examiner repeats a requirement for the Applicants to update the status of all US applications cited. The Applicants again respectfully point out that the amendment to the specification in the Applicants' Amendment & Response dated 12/07/2007 has updated that status of all patent applications disclosed (e.g., page 4, lines 16-22, and on pages 13 to 14 of the instant application). Should the Examiner believe further status update is needed, Applicants request clarification.

3. Regarding the Examiner's requirement to change the relationship of the application from a continuation to a continuation-in-part, the Applicants point out that the claims as amended herein are claims corresponding to the language in the specification as filed. Accordingly, this application is a continuation application of the prior filed application with no new matter. Specifically, claims 1, 26, 33, and 38 have been amended to recite "a pro-MS immune response." In the specification (see, e.g., p. 13, starting at line 7), the Applicants define what is meant by the term "pro-MS immune response," in a section of the specification containing definitions of terms in a manner sufficient to give one of ordinary skill in the art notice of the meaning of the term "pro-MS immune response," whether for purposes of §112, or for purposes of determining any relevance of any reference being considered as possible prior art. Therefore, the amended claims are within the scope of the specification as filed (and therefore, the claims do not constitute new matter, and the relationship of the application is as the Applicants state above).

In the alternative, Applicants note that they are entitled to define claim terms:

> "Often the invention is novel and words do not exist to describe it. The dictionary does not always keep abreast of the inventor. It cannot. Things are not made for the sake of words, but words for things." *Autogiro Co. of Am. v. United States*, 384 F.2d 391, 397

(Ct. Cl. 1967). To overcome this lag, patent law allows the inventor to be his own lexicographer. *Chicago Steel Foundry Co.* v. *Burnside Steel Foundry Co.*, 132 F. 2d 812 (7th Cir. 1943); *Stuart Oxygen Co. Ltd.* v. *Josephian*, 162 F. 2d 857 (9th Cir. 1947); *Universal Oil Products Co.* v. *Globe Oil & Refining Co.*, 137 F. 2d 3 (7th Cir. 1943), *aff'd* 322 U.S. 471 (1944).

"...inventors may act as their own lexicographers and use the specification to supply implicitly or explicitly new meanings for claim terms." *Bell Atl. Network Servs., Inc. v. Covad Communications Group, Inc.*, 262 F.3d 1258, 1268, 59 U.S.P.Q.2d 1865, 1870 (Fed. Cir. 2001).

The written description provides a context for the claims, and is appropriately resorted to "for the purpose of better understanding the meaning of a claim," *White v. Dunbar,* 119 U.S. 47 at 51 (Supreme Court 1886) and for "showing the connection in which a device is used," *McCarty v. Lehigh Valley R.R. Co.*, 160 U.S. 110, 116, 40 L. Ed. 358, 16 S. Ct. 240, 1895 Dec. Comm'r Pat. 721 (1895). The claims of a patent may incorporate parts of the written description by reference, thus "limiting the patent to the form described." *Smith v. Snow*, 294 U.S. 1, at 11 (Supreme Court 1935). A patent applicant thus has the flexibility to imbue new or old terms with a different meaning than they would otherwise have to a person of ordinary skill in the art. *See Autogiro Co. of Am. v. United States*, 181 Ct. Cl. 55, 384 F.2d 391, 397 (Ct. Cl. 1967) ("Patent law allows the inventor to be his own lexicographer."). All that is required is that the patent applicant set out the different meaning in the specification in a manner sufficient to give one of ordinary skill in the art notice of the change from ordinary meaning. *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994); *Intellicall, Inc. v. Phonometrics, Inc.*, 952 F.2d 1384, 1387-88 (Fed. Cir. 1992). Because the inquiry into the meaning of claim terms is an objective one, a patentee who notifies the public that claim terms are to be limited beyond their ordinary meaning to one of skill in the art will be bound by that notification, even where it may have been unintended. *See, e.g., Liebel-Flarsheim Co. v. Medrad, Inc.*, 358 F.3d 898, 905-09 (Fed. Cir. 2004); *Scimed Life Sys., Inc. v. Advanced Cardiovascular Sys., Inc.*, 242 F.3d 1337, 1344 (Fed. Cir. 2001).

> In a written description case, the "primary consideration is factual and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure." *Union Oil Co. of Cal. v. Atlantic Richfield Co.*, 208 F.3d 989, 996, 54 U.S.P.Q.2D (BNA) 1227, 1232 (Fed. Cir. 2000) (quoting *In re Wertheim*, 541 F.2d 257, 262, 191 U.S.P.Q. (BNA) 90, 96 (CCPA 1976)). An analysis of the adequacy of a disclosure begins with a direct comparison of the claims to the disclosure in the priority document. If the claim language is not expressly supported by the disclosure, then the language of the priority document must be analyzed for what it conveys to one skilled in the art. *Ralston Purina v. Far-Mar-Co., Inc.*, 772 F.2d 1570, 1575, 227 U.S.P.Q. (BNA) 177, 179 (Fed. Cir. 1985). The written description requirement does not dictate that the applicant describe the invention exactly. Rather, what is required is that, as of the filing date, the inventor convey with reasonable clarity to those skilled in the art that the inventor was in possession of the subject matter claimed. *See Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 U.S.P.Q.2D (BNA) 1111, 1117 (Fed. Cir. 1991).

In the present specification and claims, the term "pro-MS immune response" is defined for the relevant art by the Applicants at the time of the invention because of a phenomenon discovered by the Applicants; and which term is defined with particular meaning and in detail in the specification in a way so as to more than adequately and reasonably convey to one of ordinary and relevant skill in the art that the Applicants, at the time of the invention, were in possession of the subject matter recited in claims 1, 26, 33, and 38. Thus, the term "pro-MS immune response" appearing in the claims must be accepted to mean "pro-MS immune response" as defined in the specification, because to do otherwise would be contravening the basic principles of patent law (see, supporting principles and citations above).

> With respect to compliance with the first paragraph of section 112: "If the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more." *Chemcast Corp. v. Arco Industries*

Corp. 854 F.2d 1328 (Fed. Cir. 1988) citing *Georgia-Pacific Corp.* v. *United States Plywood Corp.*, 258 F.2d 124, 136, 118 U.S.P.Q. 122, 132 [*8] (2d Cir.) *cert. denied,* 358 U.S. 884 * * * (1958). *Shatterproof Glass,* 758 F.2d at 624, 225 U.S.P.Q. at 641.

The amendments to the claims result in the claims including the term "pro-MS immune response," defined in the specification by the inventors, and which reasonably apprises those skilled in the art of both the utilization and scope of the invention; and the language of this term is as precise as the subject matter permits. Therefore, the amended claims 1, 26, 33, and 38 are supported in the specification as filed, and the claims are commensurate with the scope of the specification and parent applications.

4. Rejection of claims 1, 20-23, 26, 29-31, 33, 36-38, 41, and 43-45 under 35 U.S.C. 112 is overcome.

Claims 21, 29, 36, and 41 have been canceled by this Amendment. Reconsideration of the rejection of claims 1, 20, 22-23, 26, 30-31, 33, 37-38, and 43-45 under 35 U.S.C. 112 first paragraph is respectfully requested for the following reasons. Claims 1, 26, 33, and 38 (and hence their respective dependent claims) have been amended to recite that the antibody is a humanized antibody or a chimeric antibody, as defined in the specification as filed (e.g., see beginning last line on page 11, through page 12, and onto lines 1-4 on page 13), and as the Examiner acknowledges is disclosed in the specification (see, item 7 of the Office Action dated 06/27/2008). The Applicants appreciate the Examiner's suggestion for a revision to the claim language.

5. Rejection of claims 1, 20-23, 26, 29-31, 36-38, 41, 43-45, and 48 under 35 U.S.C. 112 is overcome.

Claims 21, 29, 36, and 41 have been canceled by this Amendment. Reconsideration of the rejection of claims 1, 20, 22-23, 26, 30-31, 33, 37-38, 43-45, and 48 under 35 U.S.C. 112 first paragraph is respectfully requested for the following reasons. Claims 1, 26, 33, 38, and 45 (and hence their respective dependent claims) have been amended to recite that the antibody binds a

determinant selected from the group consisting of human CD19, CD20, CD21, CD22, Lym-1, and CDIM, as per the Examiner's suggestion in item 8 of the Office Action dated 06/27/2008. The Applicants appreciate the Examiner's suggestion for a revision to the claim language.

6. Rejection of claims 21, 29, 36 and 41 under 35 U.S.C. 112 is moot.

Claims 21, 29, 36, and 41 have been canceled by this Amendment.

7. Regarding the application of references the Examiner has cited as prior art, this is a continuation application entitled to the priority of the parent application for the reasons stated above in item 3 herein, and as supported by patent case law and statutes.

8. Rejection of claims 1, 20-23, 33, 36-38, 41, 43-45, and 48 under 35 U.S.C. 102 is overcome.

Claims 21, 36 and 41 have been canceled by this Amendment. Reconsideration of the rejection of claims 1, 20, 22-23, 33, 37-38, 43-45, and 48 under 35 U.S.C. 102(b) is respectfully requested for the following reasons.

Claims 1, 33 and 45 (and hence their respective dependent claims) have been amended to recite the method "consisting of." This, with the fact that these claims also recite either a "composition" or "affinity ligand" "consisting of" makes it clear that the claim language is not open ended to include administration of anti-CD19 antibody in combination with other antibodies. Likewise claim 38 (and hence, claims dependent thereon) have been amended to recite the method involves administering a "single composition" (see, e.g., p. 25, lines 6-10), and wherein the composition "consists" of an affinity ligand; making it clear that the claim language is not open ended to include administration of anti-CD19 antibody in combination with other antibodies.

The Meyer et al. reference is cited by the Examiner as a reference under §102 teach using the anti- B cell antibody Lym-1 in conjunction with therapeutic antibody or diagnostic antibody to suppress an immune response against the administered therapeutic or diagnostic antibody (p.2,

38-40). Thus, Meyer at al. teaches using anti-Lym-1 in combination with other antibodies. Further, Meyer et al. fail to teach a method for treating a pro-MS immune response.

The U.S. Court of Appeals for the Federal Circuit court has repeatedly stated that anticipation under 35 U.S.C. 102 can only be established by a single prior art reference which discloses each and every element of the claimed invention. *RCA Corp. v. Applied Digital Data Systems, Inc.*, 730 F.2d 1440, 1444, 221 U.S.P.Q. (BNA) 385, 388 (Fed. Cir. 1984); *Radio Steel & Mfg. Co. v. MTD Products, Inc.*, 731 F.2d 840, 845, 221 U.S.P.Q. (BNA) 657, 661 (Fed. Cir. 1984); *Connell v. Sears, Roebuck & Co.*, 722 F.2d 1542, 1548, 220 U.S.P.Q. (BNA) 193, 198 (Fed. Cir. 1983); *Kalman v. Kimberly-Clark Corp.*, 713 F.2d 760, 772, 218 U.S.P.Q. (BNA) 781, 789 (Fed. Cir. 1983); *SSIH Equipment, S.A. v. U.S. Int'l. Trade Comm'n.*, 718 F.2d 365, 377, 218 U.S.P.Q. (BNA) 678, 688 (Fed. Cir. 1983). In other words, the cited reference must identically disclose and describe the claimed invention for the reference to anticipate the not identically disclose and describe the claimed invention within the meaning of 35 U.S.C. 102. For example, the claimed methods of the present invention differ from Meyer et al. by administration of an antibody, versus a combination of antibodies taught by Meyer et al. Further, the Applicants claimed methods are directed to reducing a pro-MS immune response, not to suppress a host immune response to exogenous antibodies administered to an individual for therapeutic or diagnostic purposes (see p, 2, lines 7-20, and lines 38-40).

Further, for the reasons stated by the Examiner previously, the claims are being examined for the species of antibody to CD19, not Lym-1 which has been determined by the Examiner to be a separately patentable species. Why has the Examiner required the Applicants to elect a species for examination, if such species election is not applied in examination? MPEP 706.02(b) states that a rejection based on 35 U.S.C. 102(b) can be overcome by establishing that the claims are patentably distinguishable over from the reference cited as prior art; and the Examiner's species election is evidence of the claims being examined for CD19 as being patentably distinguishable over a reference citing Lym-1.

In summary, Meyer et al. does not identically disclose or describe the method recited in amended claims 1, 20, 22-23, 33, 37-38, 43-45, and 48 and therefore, the claims cannot be

anticipated by Meyer et al. under the meaning of 25 U.S.C. 102(b). Accordingly, it is respectfully requested that this rejection be withdrawn.

9. Rejection of claims 1, 20-23, 33, 36-38, 41, 43-45, and 48 under 35 U.S.C. 103 is overcome.

Claims 21, 36 and 41 have been canceled by this Amendment. Reconsideration of the rejection of claims 1, 20, 22-23, 33, 37-38, 43-45, and 48 under 35.U.S.C. 103(a) is respectfully requested for the following reasons. In the instant Office Action, the Examiner rejects claims 1, 18, 20-24, 33, 34, 36-39, and 41-48 under 35 U.S.C. 103 as being unpatentable over Meyer et al. in view of Pesando (WO 91/13974) and Arrufo et al. In making this rejection, the Examiner notes (in bold text) that the claims of the application under consideration encompass treatment using anti-CD19 antibody in combination with other antibodies. Applicants respectfully point out that claims 1, 33 and 45 (and hence their respective dependent claims) have been amended to recite the method "consisting of." This, with the fact that these claims also recite either a "composition" or "affinity ligand" "consisting of" makes it clear that the claim language is not open ended to include administration of anti-CD-19 antibody in combination with other antibodies. Likewise, claim 38 (and hence, claims dependent thereon) have been amended to recite the method involves administering a "single composition," and wherein the composition "consists" of an affinity ligand; making it clear that the claim language is not open ended to include administration of anti-CD19 antibody in combination with other antibodies. Secondly, the Examiner cites *In re Schulze* in his position that arguments of counsel cannot take place of evidence in the record. Applicants believe there is sufficient evidence in the record (e.g., in the teachings away from the claimed invention by the cited references) that the Examiner has failed to meet his initial burden of establishing a *prima facie* case of obviousness as required by patent law (The Examiner bears the burden of establishing a *prima facie* case of obviousness. *In re Rijckaert,* 9 F.3d 1531, 1532, 28 U.S.P.Q.2D (BNA) 1955, 1956 (Fed. Cir. 1993)). To advance prosecution, Applicants submitted herewith a Rule 132 Declaration with supporting evidence of nonobviousness of the claimed invention.

By virtue of his advanced degree in Immunology and Microbiology, and as a person with knowledge based on experience in the field of immunology and immunotherapy, Dr. Nelson offers a factual-based opinion on the level of ordinary skill in the art at the time of the invention. As evidence of the facts in issue, Dr. Nelson discusses 2 publications which are probative evidence of a finding of nonobviousness of the claimed invention ("Publications may, however, be evidence of the facts in issue and should be considered to the extent they are probative"; MPEP 716.02(g)). Since the claimed invention relates to immunotherapy of an aberrant immune response discovered in individuals with progressive multiple sclerosis, entered into the record is a publication describing immunotherapeutic approaches to MS existing at the time of the invention (Karussis et al., *Journal of Neurological Sciences* 153 (1998) 239-250, "Immunomodulating therapeutic approaches for multiple sclerosis"). From this publication, it is clear that one of reasonable skill in the art: (a) was focusing immunotherapeutic approaches against autoreactive T cells; (b) that depleting B cells alone was not apparent to one skilled in the art at the time of the invention as an immunomodulating therapeutic approach for multiple sclerosis; (c) that even if one were to perform total lymphoid irradiation (depleting both T and B cells), such treatment was not effective by itself but rather required treatment combination with steroids to show some beneficial effect on progression of MS; and (d) that depleting B cells may be contrary to halting progression of MS, since it was known that antibodies showed a beneficial effect (e.g., significant reduction of the rate of relapsing). Further as probative evidence, entered into the record Asakura et al., *The Journal of Neuroscience* 18(19):7700-7708. As explained by Dr. Nelson, Asakura et al. indicate that antibodies can be found which promote CNS remyelination, hence conclude that "oligodendrocyte-reactive natural autoantibodies may provide a powerful and novel therapeutic means to induce remyelination in multiple sclerosis patients." Thus as probative evidence as to the field of immunotherapeutic approaches to MS at the time of the invention, common sense of one of reasonable skill in the art at the time of the invention would not think to deplete B cells as immunotherapy of multiple sclerosis because (a) depleting B cells, as part of total lymphoid irradiation, had no beneficial effect on progression of multiple sclerosis, and in fact (b) antibodies have shown a beneficial effect on reducing progression and/or healing in multiple sclerosis. As Dr. Nelson states, not only was a pro-MS

immune response believed to be unknown to those skilled in the art at the time of the invention, further, based on what was known to one of ordinary skill at the time of the invention as to immunotherapeutic approaches to MS, it would be unexpected that B cell depletion would have a beneficial effect on an immune response underlying progressive MS.

Evidence of unexpected properties may be in the form of a direct or indirect comparison of the claimed invention with the closest prior art which is commensurate in scope with the claims. MPEP §716.02(b); see *In re Boesch*, 617 F.2d 272, U.S.P.Q. 215 (CCPA 1980) and MPEP §§ 716.02(d)-(e). Also, the Supreme Court, in *KSR International Co., v. Teleflex Inc. et al.*, 127 S. Ct. 1727, at 1734 (also 82 U.S.P.Q.2D (BNA) 1385) (hereafter, "*KSR*") reconfirmed that the *Graham* factors are the framework to be applied for the statutory language of Section 103. Hence, evidence of nonobviousness that must be considered in an analysis under §103 is (i) the prior art teaching away from the claimed invention; and (ii) the claimed invention results in unexpected properties or results. The following additional remarks below are made with reference to the amended claims herein and with respect to the cited references.

A. In summary, the Meyer et al. teaches using the anti- B cell antibody Lym-1 in conjunction with therapeutic antibody or diagnostic antibody to suppress an immune response against the administered therapeutic or diagnostic antibody (p.2, 38-40). Arrufo et al. teaches using a pan-immune cell antibody recognizing the determinant CD40 (expressed by B cells, dendritic cells, keratinocytes, monocytes, macrophages, epithelial cells, endothelial cells, fibroblasts, eosinophils, and T cells). It is well known at the time of the invention that T cells and macrophages play a major role in causing multiple sclerosis (see, e.g., the instant application at p. 2 lines 10-21, and Table 1). Thus, use of an antibody against CD40 affects T cells and macrophages, and which constitutes a treatment totally different in function and therapeutic effect than using an anti- B cell antibody for depleting B cells with associated with a pro-multiple sclerosis immune response. Pesando et al. teach a dual antibody approach, the combination of an antibody which binds to sIg on B cells and an antibody to CD19 in the form of a CD-19 specific immunoconjugate; and that only a subset of B cells possess sIg (see, for example, p. 4, lines 5-13). The combination of sIg and CD19 antibodies to form the immunoconjugate is directed to internalizing the immunoconjugate to the intracellular

compartment of targeted B cells (see p. 3, lines 13-17). None of Meyer et al., Arrufo et al., or Pesando et al. individually, or combined could result in the treatment of (i) a pro-MS immune response (which was unknown until disclosed by the instant application); (ii) reducing a pro-MS immune response by using a composition which targets specific B cell subpopulations involved in a pro-MS immune response cells using an antibody targeting a determinant selected from the group consisting of CD19, CD20, CD21, CD22, Lym-1, and CDIM. To establish *prima facie* obviousness of a claimed invention, all claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of a claim against the prior art". *In re Wilson*, 424 F.2d 1382, 1385, 165 U.S.P.Q. 494,496 (CCPA, 1970). See also, MPEP 2143.03. Due to the unobviousness of the elements of the claimed method according to the invention, the subject matter as a whole would not have been obvious to one of ordinary skill in the art at the time the invention was made.

B. A prior art reference must be considered in its entirety, i.e., as a whole, including the portions that would lead or teach away from the claimed invention. *United States v. Adams* 383 U.S.39, at 51-52 (Supreme Court 1966); *W.L. Gore & Associates, Inc. v. Garlock, Inc.,* 721 F. 2d 1540, 220 U.S.P.Q. 303 (Fed. Cir. 1983). See also MPEP 2145. Further, the references cannot be combined where the references teach away from their combination. *In re Grasselli*, 713 F.2d 731, 743, 218 U.S.P.Q. 769, 779 (Fed. Cir. 1983). See also, MPEP 2145. Each of Meyer et al., Arrufo et al., and Pesando et al. teaches away from the claimed invention. Both Meyers et al. and Pesando teach a combination of antibodies. Meyers et al. teaches using the anti- B cell antibody Lym-1 in conjunction with therapeutic antibody or diagnostic antibody to suppress an immune response against the administered therapeutic or diagnostic antibody, not to treat a pro-MS immune response or MS itself. Pesando et al. teach a dual antibody approach, the combination of an antibody which binds to sIg on B cells and an antibody to CD19 in the form of a CD-19 specific immunoconjugate; and that only a subset of B cells possess sIg. These references, either singly or combined, teach away from using a method consisting of administering a single antibody recognizing a determinant on B cells, such as CD19, to deplete B cells for reducing a pro-MS immune response.

In view of the foregoing, including the Rule 132 Declaration with publications, the invention recited in claims 1, 20, 22-23, 33, 37-38, 43-45, and 48 is patentable over the combination of Meyer et al. in view of Pesando (WO 91/13974) and Arrufo et al. within the meaning of under 35 U.S.C. 103. Accordingly, it is respectfully requested that this rejection be withdrawn.

10. In the instant Office Action, the Examiner rejects claims 20, 26, 29-31 under 35 U.S.C. 103 as being unpatentable over Meyer et al. in view of Pesando (WO 91/13974), Arrufo et al., and Turk et al. (U.S. Patent No. 5,958,409). Reconsideration of the rejection of claims 20, 26, 29-31, under 35.U.S.C. 103(a) is respectfully requested in view of the amendments to the claims, and for the reasons stated (including Rule 132 Declaration with publications, case law citations, MPEP sections, and evidence of nonobviousness) as applied to the reconsideration of the rejection under section 103 of claims 1, 20, 22-23, 33, 37-38, and 43-45, and 48 in view of the combination of Meyer et al. in view of Pesando (WO 91/13974) and Arrufo et al. The contribution of Turk et al. to the cited combination is that Turk et al. disclose a method for treating multiple sclerosis using an anti-TNF-antibody. As explained in Turk et al. (column 3, lines 12 to 21), TNF is a secreted (not determinant found on) *in vivo* by monocytes and macrophages, and possibly some T cell subpopulations; and Turk et al. describe CNS-directed antibody administration using an anti-TNF-antibody. The Examiner states "Thus, use of intrathecal antibody treatment for treating MS was known in the art," and therefore the technique is obvious. However, the standard of analysis is whether or not the invention **as a whole** is obvious in view of the **combination** of references. The combination of references fail to make the claimed invention as a whole obvious for the reasons which can be summarized as follows.

a) As supported in the Rule 132 Declaration and the references as probative evidence, it is clear that one of reasonable skill in the art at the time of the invention: (a) was focusing immunotherapeutic approaches against autoreactive T cells; (b) that depleting B cells alone was not apparent to one skilled in the art at the time of the invention as an immunomodulating therapeutic approach for multiple sclerosis; (c) that even if one were to perform total lymphoid irradiation (depleting both T and B cells), such treatment was not

effective by itself but rather required treatment combination with steroids to show some beneficial effect on progression of MS; and (d) that depleting B cells may be contrary to halting progression of MS, since it was known that antibodies showed a beneficial effect (e.g., significant reduction of the rate of relapsing and/or participated in remyelination). As Dr. Nelson states, not only was a pro-MS immune response unknown to those skilled in the art at the time of the invention, further, based on what was known to one of ordinary skill at the time of the invention as to immunotherapeutic approaches to MS (see, Karussis et al.), it would be unexpected that B cell depletion would have a beneficial effect on an immune response underlying progressive MS.

b) The Applicants respectfully point out that claims 1, and 26 (and hence their respective dependent claims) have been amended to recite the method "consisting of." This, with the fact that these claims also recite either a "composition" or "affinity ligand" "consisting of" makes it clear that the claim language is not open ended to include administration of anti-CD-19 antibody in combination with other antibodies. The Supreme Court, in *KSR International Co., v. Teleflex Inc. et al.*, 127 S. Ct. 1727, at 1734 (also 82 U.S.P.Q.2D (BNA) 1385) (hereafter, "*KSR*") reconfirmed that the *Graham* factors are the framework to be applied for the statutory language of Section 103. Hence, evidence of nonobviousness that must be considered in an analysis under §103 is (i) the prior art teaching away from the claimed invention; and (ii) the claimed invention results in unexpected properties or results. Further, the references cannot be combined where the references teach away from their combination. *In re Grasselli*, 713 F.2d 731, 743, 218 U.S.P.Q. 769, 779 (Fed. Cir. 1983). See also, MPEP 2145. Each of Meyer et al., Arrufo et al., and Pesando et al. teaches away from the claimed invention. Both Meyers et al. and Pesando teach a combination of antibodies. Meyers et al. teaches using the anti- B cell antibody Lym-1 in conjunction with therapeutic antibody or diagnostic antibody to suppress an immune response against the administered therapeutic or diagnostic antibody, not to treat a pro-MS immune response or MS itself. Pesando et al. teach a dual antibody approach, the combination of an antibody which binds to sIg on B cells and an antibody to CD19 in the form of a CD-19 specific immunoconjugate; and that only a subset of B cells possess sIg. Turk et al. disclose a method for treating multiple sclerosis using an anti-TNF-antibody; TNF is a

secreted *in vivo* by monocytes and macrophages, and possibly some T cell subpopulations; not a determinant found on B cells. These references, either singly or combined, teach away from using a method consisting of administering a single antibody recognizing a determinant on B cells, such as CD19, to deplete B cells for reducing a pro-MS immune response.

c) None of Meyer et al., Arrufo et al., Pesando et al., or Turk et al., individually or combined, could result in the treatment of (i) a pro-MS immune response (which was unknown until disclosed by the instant application); (ii) reducing a pro-MS immune response by using a composition which targets specific B cell subpopulations involved in a pro-MS immune response cells using an antibody targeting a determinant selected from the group consisting of CD19, CD20, CD21, CD22, Lym-1, and CDIM. To establish *prima facie* obviousness of a claimed invention, all claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of a claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 U.S.P.Q. 494,496 (CCPA, 1970). See also, MPEP 2143.03. Due to the unobviousness of the elements of the claimed method according to the invention, the subject matter as a whole would not have been obvious to one of ordinary skill in the art at the time the invention was made.

In view of the foregoing, the invention recited in claims 20, 26, and 29-31 is patentable over the combination of Meyer et al. in view of Pesando (WO 91/13974), Arrufo et al., and Turk et al. within the meaning of under 35 U.S.C. 103. Accordingly, it is respectfully requested that this rejection be withdrawn.

In view of the claim amendments, the Rule 132 Declaration and references showing probative evidence, and the remarks including the citation of supporting case law and MPEP sections, Applicants believe the claims now meet the requirements of patentability under 35 USC §§ 112, 102 and 103.

Respectfully submitted,

Dated: September 19, 2008

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application: Barbera-Guillem et. al. Group Art Unit: 1644

Application No.: 10/626,213 Examiner: Ronald Schwadron, Ph.D.

Filing Date: 07/24/2003 Docket No.: 26983-133

Conf. No.: 9675

Title: METHODS AND COMPOSITIONS FOR IMMUNOTHERAPY OF B CELL INVOLVEMENT IN PROMOTION OF A DISEASE CONDITION COMPRISING MULTIPLE SCLEROSIS

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Dear Sir,

I, M. Bud Nelson hereby declare the following:

1. I am a citizen of the United States.

2. I am a co-inventor of claims, presently under consideration in the above-identified application, through my scientific contributions as an employee of BioCrystal Ltd. (the assignee of the captioned application).

3. I am very familiar with the disclosure of the above-identified application, and participated in drafting the application.

4. I have earned the following four scientific degrees: Bachelors degree in Biology, Bachelors degree in Medical Technology, and a Master's degree and Ph.D. degree in Immunology and Microbiology.

5. At BioCrystal Ltd., I was heavily involved in the study of immunology and immunotherapy, as evidenced by the following co-authored publications:

a) Nelson et al., "Tumor cells express FcγRI which contributes to tumor cell growth and a metastatic phenotype"; *Neoplasia* 3(2):115-124, 2001.

b) Barbera-Guillem et al., "Promotion of tumor invasion by cooperation of granulocytes and macrophages activated by anti-tumor antibodies"; *Neoplasia* 1(5):453-460, 1999.

c) Nyhus et al., "IgG recognizing shed tumor-associated antigens can promote tumor invasion and metastasis"; *Cancer Immunology Immunotherapy* 50:361-372, 2001.

d) Barbera-Guillem et al., "B lymphocyte pathology in human colorectal cancer"; *Cancer Immunology Immunotherapy* 48:541-549, 2000.

6. I have approximately a one percent interest in the shares of privately-held stock of BioCrystal Ltd. I also serve as a paid consultant to BioCrystal, Ltd. until Dec. 2008 on patent matters.

7. Prior to the priority date of the above-identified application, 20 September 1999, and as shown in Table 1 of the patent application (end of paragraph 0005 as published), the then current therapies for multiple sclerosis ("MS") were directed to T cells, and to macrophages and microglial cells.

8. The claims pending in the present application, including the amendments filed concurrently with this declaration, are directed to a method of immunotherapy of MS, and more specifically, to treatment of an immune response to a specific antigen, as evidenced by certain subpopulations of B cells, believed to promote the progression of MS.

9. Related to the issues of obviousness or non-obviousness of the claims in the above-identified application, and as evidence of probative value of what was known to those of reasonable skill in the art at the time of the invention in terms of immunotherapeutic approaches to MS, I attach a review by Karussis and Abramsky, published in *Journal of Neurological Sciences* 153 (1998) 239-250, entitled "Immunomodulating therapeutic approaches for multiple sclerosis" ("Karussis et al."; attached hereto as Exhibit A). As stated in the Introduction (Karussis et al., p. 239), in MS plaques and in the experimental animal model of MS (Experimental autoimmune encephomyelitis or EAE), T cells, mainly of the CD-4 phenotype, are activated against myelin proteins resulting in the pathology observed in MS. Activated macrophages also are active in this inflammatory process. Thus, Karussis

et al. reviews the conventional and potentially new therapeutic approaches directed to MS mediated by the autoreactive or "myelin-reactive" T cells. "Therefore, alternative immunomodulating approaches to the treatment of MS (and autoimmunity in general) should preferably be directed towards activation of normal down-regulatory circuits or towards induction of specific unresponsiveness (tolerance/anergy) of myelin-reactive T cells" (Karussis et al., p. 241, column 2).

10. As discussed in the "Conventional treatments" section (Karussis et al., p. 241, column 1), even total lymphoid irradiation (depleting T cells and B cells) did not work by itself to effect a beneficial response in MS in the progression of MS, but only when combined with steroids has some beneficial effect to prevent the deterioration rate in progressive MS. Thus, based on what was known at the time of the invention about immunomodulatory therapy of MS, as summarized by Karussis et al., one of reasonable skill in the art with common sense would not recognize lymphocyte depletion, and particularly B cell depletion, as a treatment which could be of some benefit to prevent progression of MS disease. However, unexpected as compared to the results summarized by Karussis et al., we (the Applicants of the present claimed invention) describe a method for depleting B cell populations associated with a pro-MS immune response, in individuals with progressive MS, as first described by us in the present application and recited in the claims.

11. Further, Karussis et al. describe that a recent "controlled study with monthly IVIG injections (intravenously-administered immunoglobulin therapy) has shown beneficial effects in patients with relapsing-remitting MS (Fazekas et al., 1997), inducing a reduction of 59% in the relapse rate"; and "a statistically significant difference between the placebo and IVIG tested groups in the progression of disability" (Karussis et al., p. 241, first column). Thus, based on what was known at the time of the invention about immunomodulatory therapy of MS, as summarized by Karussis et al., one of reasonable skill in the art with common sense could reasonably conclude that antibodies could play a regenerative or beneficial role in preventing progression of MS disease. This conclusion, however, would teach away from the claimed depletion of antibody-producing B lymphocytes to reduce a pro-MS immune response.

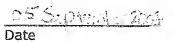
12. As further evidence that antibodies (and hence, the B cells which produce the antibodies) were believed to have a beneficial role in therapy of MS, I attach a paper by Asakura et al., published in *The Journal of Neuroscience* 18(19):7700-7708, entitled "Targeting of IgMκ Antibodies to Oligodendrocytes Promotes CNS Remyelination" ("Asakura et al."; attached hereto as Exhibit B). Using an experimental model of MS, Asakura et al. conclude that "these studies indicate that oligodendrocyte-reactive natural autoantibodies may provide a powerful and novel therapeutic means to induce remyelination in multiple sclerosis patients" (Asakura et al., last line of Abstract).

13. I have reviewed the references cited by the Examiner in this case. Meyer et al. highlights a problem that murine antibodies are recognized as foreign proteins in a human body (Meyer, p. 2, lines 27-29). Therefore, when such a foreign antibody is used as a diagnostic antibody or therapeutic antibody to treat humans, such foreign antibody can be recognized by the host's B lymphocytes which themselves may produce antibodies to neutralize the therapeutic antibody or diagnostic antibody (see, Meyer, p, 2, lines 7-20). Meyer et al. teach a method to suppress an immune response to a foreign antibody (therapeutic antibody or diagnostic antibody) by administering both an anti- B cell antibody Lym-1 and the therapeutic antibody or diagnostic antibody to suppress an immune response against the administered therapeutic or diagnostic antibody (Meyer, p.2, 38-40). The Lym-1 antibody is also a murine antibody (Meyer, p. 3, lines 22-25). Meyer et al. does not teach use of the Lym-1 antibody to treat a pro-MS immune response that promotes progression of MS, but rather teaches the use of Lym-1 antibody in conjunction with a diagnostic antibody or therapeutic antibody used in the treatment of autoimmune diseases to prevent a host reaction against the administered, foreign diagnostic antibody or therapeutic antibody.

14. Further, Meyer et al provides no description for to one skilled in the art to understand what these diagnostic or therapeutic antibodies are (what is their target), or how they are used (how much, when, where, administered) to treat MS. Thus, it is clear that Meyer et al. reference does not teach treatment of MS (versus treatment of a host reaction against foreign antibody) with Lym-1 antibody. In view of the fact that antibodies (IVIG or oligodendrocyte-reactive natural autoantibodies) can be helpful to prevent progression of MS, and in view of the fact that we, the Applicants

in the subject application, are believed to first describe a specific immune response (pro-MS immune response) and resultant abnormal phenotype of B cells present in individuals with progressive MS, one of reasonable skill in the art would not at the time of the invention (a) recognize or expect lymphocyte depletion, and particularly B cell depletion, as a treatment which could be of some benefit to prevent progression of MS disease (see points above and subject application for supporting factual basis), or (b) combine Meyer et al. in view of Pesando,and Arrufo et al., or further in view of Turk et al., in an attempt to obtain the claimed invention.

I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.



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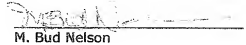

M. Bud Nelson

Exhibit A

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Immunomodulating therapeutic approaches for multiple sclerosis

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Abstract

In this review we delineate the rationale for immunotherapy in multiple sclerosis and describe the various levels at which immune intervention, according to a modern model of the immune system organization, is feasible. Current and future immunosuppressive and immunomodulating therapeutic approaches at the level of antigen presentation and at the lymphocyte and cytokine network levels are discussed. © 1998 Elsevier Science B.V.

Keywords: Multiple sclerosis (MS); Experimental autoimmune encephalomyelitis (EAE); Immunomodulation

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized histologically by focal lymphocyte and macrophage infiltrates and demyelination and, clinically, by multi-focal neurological signs (Hafler and Weiner, 1989; Raine, 1994). In acute MS plaques, activated T cells secreting regulatory cytokines and expressing growth factor receptors for IL-2, as well as activated class II MHC positive macrophages, are present. The active inflammatory process is confined to the white matter of the CNS, without affecting either the peripheral nervous system or other organs (Hafler and Weiner, 1989; Raine, 1994). There are two main explanations for the CNS inflammation: the white matter (possibly the oligodendrocytes that produce myelin) is infected by a virus or other infectious agent and the infiltrating cells are targeted to the infectious agent. Alternatively, the infiltrating cells are of an autoimmune nature and attack normal myelin proteins, such as myelin basic protein (MBP) and proteolipid protein (PLP). Although either hypothesis is reasonable, it seems more likely that the role of any putative infectious agent is to trigger/drive the autoimmune process, rather than to serve as the primary target of infiltrating cells.

It is widely accepted that in MS, the putative autoimmune process is initiated in the peripheral immune system. Following activation by the macrophages/APCs (antigen presenting cells), the lymphocytes, mainly of the helper (CD-4)-subtype, that express the specific for the myelin antigens T-cell receptor (TCR), proliferate and begin to express on their membranes, adhesion molecules/markers of activation, which help them to extravasate to the site of inflammation into the CNS (Wilcox et al., 1990; Dore et al., 1993; Sharief et al., 1993; Svenningsson et al., 1993; Tsukada et al., 1993a,b,c, 1994; Washington et al., 1994). After the stage of clonal expansion, T-helper cells further differentiate, to become either TH-1 helpers (that provide help to cytotoxic or CD-4 T-cells-positive feedback-) producing IL-2, IL-12, TNF-a and IFN-γ (pro-inflammatory cytokines), or TH-2 helpers, that provide help mainly to B-cells and produce IL-4, IL-6 and IL-10 (Bottomly, 1988; Erb et al., 1991; Romagnani, 1991). In MS, the lymphocytes that are mainly involved in the inflammatory process are of the TH-1 phenotype.

Experimental autoimmune encephalomyelitis (EAE) is an animal model for immune-mediated demyelination. It is induced by injecting homogenates of whole spinal cord or the myelin proteins MBP and PLP. The pathologic picture of EAE, especially in the chronic relapsing form, is very similar to MS. EAE is mediated by T cells, mainly of the CD-4 phenotype, that react to myelin proteins (Bernard and Carnegie, 1975; Vanderbark et al., 1975; Wisniewski and Keith, 1977; Lublin et al., 1981; Paterson and Day, 1981; Fritz et al., 1982; Traugott et al., 1985; Cross et al., 1987; Matsumoto and Fujiwara, 1987; Minagawa et al., 1987; Namikawa et al., 1987; Teuscher et al., 1987; Tabira, 1989; Bouwer et al., 1990; Sobel et al., 1990;

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Cross et al., 1991; Jones et al., 1992; Martin and McFarland, 1995). Although it has long been argued whether EAE is a good model for MS, due to their immunologic and pathologic similarities EAE is widely used in the investigation of various therapeutic approaches to MS.

2. Conventional treatments

Conventional therapeutic strategies for MS-as for other autoimmune diseases-are based mainly on immunosuppressive modalities aimed at reducing the proliferation of autoreactive lymphocytes (Elison and Myers, 1978; Lisak, 1988; Kappos, 1988; Weiner and Hafler, 1988; Kappos et al., 1990). However, most of the immunosuppressive treatments have only shown moderate efficacy in halting the progression of the disease and in addition, are usually associated with cumulative and toxic side effects (Rudge, 1988; Silberberg, 1988).

The most widely used treatment, especially during an acute relapse of MS, is methylprednisolone or other corticosteroid preparations (Rose et al., 1970; Abbruzzese et al., 1983; Barnes et al., 1985; Thompson et al., 1989; Barkhof et al., 1991; Beck et al., 1992; Miller et al., 1992; Beck et al., 1993; Kupersmith et al., 1994). Steroids exert various effects on the immune system, such as inhibition of immune activation and T-cell proliferation (Kupersmith et al., 1994), reduction of antibody production and of adhesion molecules expression, but most importantly intravenously administered steroids appear to reduce the permeability of the blood–brain barrier (Barkhof et al., 1991; Miller et al., 1992) as evidenced by the reduction in Gd-DTPA enhancement of an acute lesion in the MRI. However, this effect is short lived and new enhancing lesions reappear within weeks following treatment with steroids (Miller et al., 1992). Results from the National Cooperative ACTH study, revealed that corticotropin (ACTH) may hasten recovery at one month after treatment compared with placebo (Rose et al., 1970). Other studies have shown comparable or superior results with methylprednisolone (Abbruzzese et al., 1983; Barnes et al., 1985; Thompson et al., 1989). More recent data from the Optic Neuritis Treatment Trial, showed that intravenous methylprednisolone is superior to oral prednisone for the treatment of acute optic neuritis (Beck et al., 1992, 1993). However the long term effect of both treatment regimens on the visual acuity was marginal and there was no significant 'protection' against later development of definite MS (Beck et al., 1992, 1993). In patients with chronic progressive MS, repeated i.v. infusions with methylprednisolone failed to reveal any inhibition of that disease progression (Whitham and Bourdette, 1989). These data indicate that although steroids may exert a transient beneficial effect during the acute MS-relapse, they don't alter significantly the natural history of the disease; therefore the extensive use of steroids does not seem justified. In addition, chronic steroid administration may induce several adverse effects, like osteoporosis, aseptic bone necrosis, hypertension, hyperglycaemia, cataracts and psychotic events.

Several other immunosuppressive treatments have been tried over the last decade, but with only marginal efficacy. Azathioprine, an antimetabolite with broad spectrum immunosuppressive effects, was tested in several clinical trials (Kappos et al., 1988; Ellison et al., 1988; The British and Dutch Multiple Sclerosis Azathioprine Trial Group, 1988; Kappos et al., 1990; Goodkin et al., 1991; Steck et al., 1990). In the largest controlled trial (The British and Dutch Multiple Sclerosis Azathioprine Trial Group, 1988), it was shown to reduce the rate of progression in three years; in this study, the worsening in the EDSS scale was 0.62 in the azathioprine group as compared to 0.80 in the placebo-treated patients. A meta-analysis of the seven randomised controlled blinded trials with azathioprine, showed a mild beneficial effect (reduction in the worsening of EDSS score by 0.22 points) (Yudkin et al., 1991) after two years of treatment.

Cyclophosphamide, an alkylating agent, was also tested in several studies (Gonsette et al., 1977; Hauser et al., 1983; Myers et al., 1987; Carter et al., 1988; Likosky, 1988; The Canadian Cooperative Multiple Sclerosis Study Group, 1991; Hafler et al., 1991; Weiner et al., 1993). The Northeastern Cooperative Treatment Group reported a benefit from cyclophosphamide 'boosters'. However the difference in stabilization rates (38 vs 24% in 24 months) was small and had disappeared by 36 months (Weiner et al., 1993). On the other hand, the Canadian Cooperative Multiple Sclerosis Study failed to show any effect of cyclophosphamide in slowing the progression of the disease (The Canadian Cooperative Multiple Sclerosis Study Group, 1991). In the same study, plasmapheresis also did not reveal any beneficial effect on MS. Therefore, the use of cyclophosphamide for the treatment of progressive MS has remained quite controversial.

Methotrexate, an antimetabolite, at a low dose, was recently reported to have a mild beneficial effect on the progression of disability, as revealed by the non-conventional 'nine-hole test' for the dexterity of the upper extremities (Goodkin et al., 1995). However, the effect of this treatment was less pronounced, when changes in the 'traditional' measures, as the EDSS score and the Ambulation Index were used as outcomes (Goodkin et al., 1995). The effect of methotrexate on the MRI activity was also marginal in this trial. Another trial with methotrexate (Currier et al., 1993) showed some reduction in the relapse rate but no benefit in patients with progressive disease.

Cyclosporin-A was studied in a multicentre double blind clinical trial involving 547 patients with chronic progressive MS (The Multiple Sclerosis Study Group, 1990) and was found to mildly reduce the progression rate and to delay the time to becoming 'wheelchair-bound'. However the high rate of hypertension and impaired renal function,

induced by this treatment, limits its use (Rudge, 1988). A more recent immunosuppressive agent, cladribine, has shown some promising effects in progressive MS patients (Sipe et al., 1994; Beutler et al., 1996) but these results remain to be confirmed by an ongoing phase III trial.

All of the above mentioned immunosuppressive treatments are associated with considerable side effects, mainly due to the induction of bone marrow suppression, with resulting increased risk for infections and thrombocytopenia and the potential risk for the development of malignancies. Therefore, their use in MS, in light of their marginal efficacy should be cautious.

Total lymphoid irradiation (TLI), a radical immunosuppressive modality, was previously reported to inhibit the progression of MS (Cook et al., 1986). However, a more recent trial from the same group, showed that only the combination of TLI with steroids has some beneficial effect, preventing the deterioration rate in progressive MS (Cook et al., 1995). This treatment is associated with substantial risk due to the extensive immunosuppression.

The use of treatments that mainly affect antibody production, like plasmapheresis and high dose immunoglobulins (IVIG), is also controversial. While two controlled studies failed to reveal any efficacy of plasmapheresis (Tindall et al., 1982; The Canadian Cooperative Multiple Sclerosis Study Group, 1991), there are data from at least one open-controlled study, indicating some efficacy of IVIG in reducing the number of relapses in relapsing-remitting MS patients (Achiron et al., 1992). However, two other studies, failed to show any efficacy of this treatment in patients with relapsing-remitting or progressive MS (Cook et al., 1992; Francis et al., 1994). MRI monitoring in the later trial did not reveal any effect of IVIG treatment in preventing the appearance of new lesions in the brain (Francis et al., 1994). A pilot study examining the issue of recovery of apparently fixed, irreversible neurological deficits, by IVIG treatment, showed that this treatment may enhance recovery in optic neuritis patients, raising the possibility of induction of remyelination (Rodriguez and Lennon, 1990; Rodriguez, 1991; Van Engelen et al., 1992). Recently, a controlled study with monthly IVIG injections, has shown beneficial effects in patients with relapsing-remitting MS (Fazekas et al., 1997), inducing a reduction of 59% in the relapse rate. There was also a statistically significant difference between the placebo and IVIG treated groups in the progression of disability (changes in EDSS score), but the effects on MRI activity were not evaluated in this trial.

3. New immunomodulator therapeutic approaches

Recent theories advocating the importance of immune networks for the preservation of an intact 'self,' have provided a new insight into the pathogenesis of autoimmune diseases. It seems that autoreactive cells and antibodies present, even in healthy individuals, are prevented from inducing autoimmunity by downregulatory circuits (Lieder et al., 1988; Cohen and Young, 1991). Disruption of these delicate balances, or defects in the normal immunoregulatory mechanisms, may be involved in the pathogenesis of autoimmunity. Several reports of defective natural killer (NK) cell activity and reduced proportions of suppressor or suppressor–inducer cells in patients afflicted with autoimmune diseases (Merrill et al., 1982; Neighbour et al., 1982; Rose et al., 1985, 1988; Chofflon et al., 1989; Ilonen et al., 1990; Zaffaroni et al., 1990, 1991; Porrini et al., 1992; Eoli et al., 1993; Khoury et al., 1994; Calopa et al., 1995; Crucian et al., 1995; Bongioanni et al., 1996; Gordon et al., 1996), lend support to this theory. Therefore, alternative immunomodulating approaches to the treatment of MS (and autoimmunity in general) should preferably be directed towards activation of normal downregulatory circuits or towards induction of specific unresponsiveness (tolerance/anergy) of myelin-reactive T-cells.

There are several levels at which it is possible to interfere with the development of the auto-immune process in MS.

3.1. Antigen presentation

The autoimmune inflammatory process is initiated after the presumed auto-antigen (MBP, PLP or MOG) is presented to T-lymphocytes by macrophages or other antigen presenting cells (APCs) (Fig. 1). For full activation/immunization, the lymphocyte has to receive two signals: (a) one provided by the MHC-antigen complex, which binds to the specific TCR (T-cell receptor), and the second (b) supplied by the B7-CD-28 (adhesion molecule) complex. In the absence of the secondary costimulatory signal, the lymphocytes fail to produce the T-cell growth factor IL-2 and, therefore, antigen presentation leads to anergy of the lymphocytes towards the specific antigen (Janeway, 1993). There are also other accessory molecules, like the LFA-3/CD-2 complex, that enhance lymphocyte reactivity in a

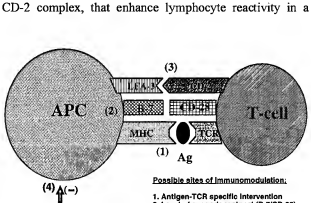

Fig. 1. Antigen presentation to T-cells: Possible sites of immune intervention.

non-specific manner. The latter represent a second pathway (independent of the TCR-pathway) for T-cell activation (Suthanthiran and Strom, 1994).

At this level therapeutic intervention can be induced by:

(i) *Tolerization towards the specific myelin auto-antigens*. This can be achieved by oral tolerization techniques (Khoury et al., 1990; Whitacre et al., 1991; Miller et al., 1993; Weiner, 1993; Sabbagh et al., 1994), immunization with peptides/parts of the specific TCR (as for example those that react against MBP or PLP) (Howell et al., 1989; Jung et al., 1993; Vandenbark et al., 1993), T-cell vaccination (Ben-Nun and Cohen, 1981, 1982; Cohen et al., 1983; Lieder et al., 1988; Beraud, 1991; Hafler et al., 1992; Zhang and Raus, 1994a), immunization with altered myelin antigen epitopes (Nicholson et al., 1995) and blockage/antagonism of the MHC/TCR complex (by proteins mimicking myelin antigens or equivalent polymers, such as co-polymer-1, COP-1) (Teitelbaum et al., 1973; Keith et al., 1979; Baumhefner et al., 1988; Bornstein et al., 1988a,b; Teitelbaum et al., 1992).

A phase II study, showing the therapeutic potential of oral tolerization with myelin antigens in patients with relapsing–remitting MS has already been reported (Weiner, 1993). In this trial, six out of fifteen patients treated with oral myelin had at least one major exacerbation of MS, as compared with twelve out of fifteen in the placebo-treated group. This approach represents an antigen-specific therapeutic modality, where the antigen-driven peripheral immune tolerance is acquired by mechanisms inducing deletion, inactivation (anergy) or active suppression of the antigen-reactive clones. A larger phase III trial with oral myelin has been recently completed, by the same group. Preliminary results from this trial do not show any significant difference in the relapse rate between the placebo and oral myelin (Myloral) treated MS patients.

Attempts have also been made to induce tolerance by immunization with TCR peptides/parts of those TCRs usually found with greater frequency (e.g. the Vb-8.2, Vb-5.2 and Vb-6.1 TCR) (Vandenbark et al., 1993; Whitham et al., 1993) in the encephalitogenic lines in the EAE model and in some MS patients. In this way, neutralizing antibodies or anti-idiotypic T-cells are induced which, in turn, block the autoreactive T-cells. This treatment succeeded in inhibiting EAE in the Lewis-rat model and has been already applied to some MS patients (Vandenbark et al., 1993). In patients immunized with Vb-5.2 peptides, a reduction in the frequency of MBP-reacting cells and an amplification of anti-TCR reacting cells was observed. However, this approach has certain drawbacks, since there is a great heterogeneity of TCRs among myelin-reactive T-cells in MS patients (Hiller et al., 1992; Ransohoff, 1992; Gold et al., 1995; Olive, 1995).

In order to overcome this obstacle, Cohen and Ben-Nun in their pioneer work (Ben-Nun and Cohen, 1981, 1982; Cohen et al., 1983) on T-cell vaccination, attempted to vaccinate EAE-animals with attenuated (by irradiation) encephalitogenic T-cell lines and showed that this treatment effectively suppressed EAE. The investigators suggested to remove a sample of T-cells from patients suffering from MS, irradiate them and then to reinject them into the same patients. In this way, an anti-idiotypic reaction could be induced which would be specific to each individual. However, even in the same patient different T-cell clones bearing variable TCRs may be activated/expanded at various stages of the disease, during MS relapses. Phase I trials have recently shown that this treatment is technically feasible (Hafler et al., 1992; Zhang and Raus, 1994a,b).

Another approach, aimed at blocking/antagonizing the MHC/TCR complex has been to use analogues of myelin antigens. Preliminary data indicate that immunization with modified MBP or PLP-peptides protects mice from developing EAE (Karin et al., 1994; Nicholson et al., 1995). This modality is still experimental.

COP-1 ('Copaxone', Teva, Israel) is a synthetic co-polymer, composed of alanine, glutamine, lysine and tyrosine, with some immunologic similarities to the MBP molecule (as well as to other myelin antigens), without itself being encephalitogenic. After finding that it inhibits EAE (Teitelbaum et al., 1973; Keith et al., 1979), COP-1 was successfully tested in two double-blind trials in patients with relapsing–remitting MS (Bornstein et al., 1988a,b; Johnson et al., 1995). In the latter two-year double-blind trial carried out in the USA, and involving 251 patients with relapsing–remitting MS, Copaxone induced a 29% reduction in relapse rate (1.19 over two years vs 1.68 in the placebo group). The proportion of patients free of relapse or progression of disability at two years, was no different between the two groups, nor was the time to first relapse in the study. MRI monitoring was performed in one of the centres and showed only marginal (and not statistically significant) inhibition of the 'MRI-activity' in the COP-1 treated patients. Adverse effects were usually mild, including mainly localized, injection-site reactions (at least once in 90% of patients) and a systemic reaction occurring within moments of COP-1 administration (associated with chest pain, palpitations or dyspnea lasting up to 30 min) in 15% of patients. COP-1 is presumed to block the MHC/TCR complex and to down-regulate the presentation of antigens, such as MBP, PLP and MOG, to T-cells.

(ii) *Blocking/inhibition of the secondary signal and induction of anergy*. This can be achieved by using antibodies which bind to the adhesion molecules, especially B7 and CD-28 (De et al., 1995; Perrin et al., 1995). It was shown in a diabetic model in transgenic mice, that only those animals expressing the B-7.1 subtype on the pancreatic islet cells and having T-cells expressing a TCR that recognizes antigens of pancreatic islet cells, fully developed diabetes. Early upregulation of B-7.1 expression was recently demonstrated in plaques of patients with MS (Windhagen et al., 1995).

(iii) *Blocking accessory signals*. This can be achieved

by the use of antibodies which bind to the CD-2 molecule (Jung et al., 1995) and thereby inhibit the alternative-bystander T-cell activation pathway. Phase I studies are already being performed in this direction. However, this approach has certain drawbacks, since it induces non-specific lymphocyte suppression. Moreover, repeated injection of monoclonal (mouse) antibodies induces the production of human anti-mouse antibodies which may neutralize the effect of the anti-CD-2 monoclonal antibodies.

(iv) *Downregulation of macrophages/APC* through utilization of cytokines or substances that downregulate antigen processing and presentation, such as IFN-β (Ling et al., 1985; Panitch, 1992; The IFNB Multiple Sclerosis Study Group, 1993; Panitch and Bever, 1993; Silberberg, 1994; Brod et al., 1995; Wilson, 1995), IL-10 (Fiorentino et al., 1991), TGF-β (Racke et al., 1992), PGE-2 and free radicals. This can also be achieved by inhibition of the pro-inflammatory cytokine IFN-γ, which upregulates MHC-expression and, therefore, enhances the ability of APCs to activate lymphocytes. Surprisingly enough, administration of anti-IFN-γ monoclonal antibodies caused a deterioration in EAE (Voorthuis et al., 1990; Duong et al., 1992; Lublin et al., 1993). This finding illustrates the complexity of the immune networks involved in the early development of an immune reaction.

Both IL-10 and TGF-β were shown to suppress EAE (Racke et al., 1991, 1993; Crisi et al., 1995; Rott et al., 1994), whereas indornethacin (which reduces PGE-2) may enhance the disease (Ovadia and Paterson, 1982). The above treatments are apparently supposed to downregulate APCs. Recently, a new synthetic immunomodulator, linomide, was found to downregulate antigen presentation, probably through overactivation of macrophages, with a resulting increase in free radicals and PGE-2 production and auto-downregulation of APCs (Lehmann et al.). We have shown that linomide is one of the most potent agents in the suppression of acute and chronic-relapsing EAE (Karussis et al., 1993a,b).

3.2. Lymphocyte network interactions (Fig. 2)

Following antigen presentation by macrophages (APCs), lymphocytes (mainly of the helper CD4-subtype) expressing the specific for the antigen TCR further differentiate, becoming either TH-1 cells, producing IL-2, IL-12, TNF-a and IFN-γ (pro-inflammatory cytokines), or TH-2 cells, secreting IL-4, IL-6 and IL-10 (Bottomly, 1988; Fiorentino et al., 1989; Bradley et al., 1991; Erb et al., 1991; Goldman et al., 1991; Romagnani, 1991; Cua et al., 1995; Khoruts et al., 1995; Kuchroo et al., 1995). As mentioned above, in MS, the lymphocytes involved in the inflammatory process are mainly of the TH-1 phenotype. Therefore, a 'shift' towards the TH-2 subtype (Kuchroo et al., 1995) is desirable and may lead to downregulation (or at least to a relative decrease) of the TH-1 lymphocytes, with a resulting reduction in inflammation. Such a shift may be

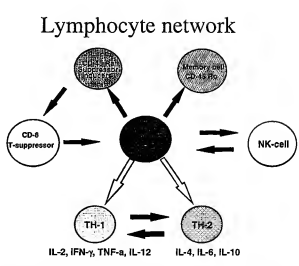

Fig. 2. Lymphocyte network: Multiple interactions between various lymphocyte subpopulations.

achieved by oral tolerization, by cytokines such as IFN-β, IL-4 and IL-10 and, possibly, by immunomodulating substances like linomide.

CD-4 lymphocytes are also functionally and phenotypically divided into two subpopulations: 'memory' cells which, after the initial encounter with the antigen, are rapidly induced to react/proliferate following a second exposure. These cells express the CD-45 RO surface marker on their membranes. The second subpopulation is of the CD 45-RA phenotype, and consists of cells which function as 'suppressor–inducers' (Sanders et al., 1987, 1988; Bradley et al., 1991; Jensen et al., 1991; Clement, 1992, Qin et al., 1993). There is probably a cyclic relationship between CD4 subpopulations: naive CD45RA cells convert to CD45RO memory cells upon antigen stimulation (Rothstein et al., 1990, 1991; Yamada et al., 1992), but without continuous antigen stimulation they lose their CD45RO expression and revert to long-lived CD45RA cells (Rothstein et al., 1990, 1991; Yamada et al., 1992). These CD45RO lymphocytes represent activated memory cells and they are probably identical to the CD29+ lymphocytes expressing on their surface the b-chain of the VLA-antigens (b1 integrins) (Pilarski et al., 1991) which is upregulated to facilitate adhesion to endothelial cells and subsequent passage into sites of inflammation. In MS and in other autoimmune diseases, like rheumatoid arthritis (RA), Guillain–Barre syndrome (GBS) and systemic lupus erythernatosous (SLE) (Rose et al., 1985, 1988; Chofflon et al., 1989; Ilonen et al., 1990; Zaffaroni et al., 1990, 1991; Porrini et al., 1992; Eoli et al., 1993; Khoury et al., 1994; Calopa et al., 1995; Crucian et al., 1995; Bongioanni et al., 1996; Gordon et al., 1996) the CD45RA cells are usually downregulated, especially during the active phases (relapses) of the disease. In a longitudinal study it was shown that newly diagnosed cases with SLE have initially a higher proportion of CD45RA cells, which, as the disease progresses, are

reduced and a shift towards the CD45RO phenotype takes place (Gordon et al., 1996). This shift seems to be the result of conversion of resting cells into activated memory lymphocytes. However, since the CD45RA cells are found in lower numbers in patients with autoimmune diseases as compared to age-matched healthy individuals, this finding may also indicate that loss of suppressor function could be, at least partially, related to the immunopathogenesis of autoimmunity in general; a low proportion of suppressor–inducer cells could permit autoreactive clones to carry out an autoimmune attack. Therefore, an increase in the CD-45 RA/CD-45 RO ratio should be an immunotherapeutic target in MS and other autoimmune diseases.

Another cell population that may be involved in the delicate cell-interactions in this network, is composed of NK (natural killer) cells, which are a part of the 'natural/non-specific immunity' (against viruses and malignant cells). NK-activity is reportedly reduced in MS and other autoimmune diseases (Merrill et al., 1982; Neighbour et al., 1982). NK cells can downregulate antigen presentation and the early phases of lymphocyte stimulation (Abruzzo and Rowley, 1983). Dentritic cells, which are important APCs, can be the target of NK-cells, especially after they have interacted with the antigen (Shah et al., 1985). Recently, a novel NK cell population expressing both NK and T-cell markers was described (Yoshimoto and Paul, 1994; Vicari and Zlotnik, 1996). These NK 1.1 cells produce upon activation large quantities of IL-4 which, in turn, induces a 'shift' of T-lymphocytes towards the TH-2 phenotype. Therefore, enhancement of NK-cell activity with immunodulators, may also be desirable in the regulation of MS.

In a recently completed double-blind pilot trial in patients with secondary progressive MS, we showed that the new synthetic immunomodulating agent, linomide, inhibits the activity of the disease (as indicated by the reduced number of active lesions in the MRI follow-up) and tends to stabilize/improve disability (Karussis et al., 1996a). Preliminary immunological data indicate that linomide upregulates the NK-cells and the CD-45 RA lymphocytes (Karussis et al., 1996b).

3.3. Cytokine network

During the inflammatory process, several peptidic substances (lymphokines or cytokines) are excreted by the lymphocytes. These are important for inter-cell 'communication'. Some of them, such as IFN-γ, IL-2, IL-12 and TNF, are pro-inflammatory, others (IL-4, IL-6, IL-10, IFN-β, and TGF-β) suppress inflammation (Bottomly, 1988; Erb et al., 1991; Romagnani, 1991; Mosman and Sad, 1996). Anti-TNF antibodies or soluble TNF receptors, and the cytokines IL-10 and TGF-β were shown to inhibit EAE (Racke et al., 1991, 1992; Samina et al., 1992; Racke et al., 1993; Crisi et al., 1995; Selmaj and Raine, 1995) and may be candidates for future immunotherapeutic approaches.

But the most important progress in this direction has been made with the cytokine IFN-β. IFN-β effectively suppressed EAE (Abreu, 1982, 1985). IFN-β inhibits T-cell activation (Rudick et al., 1993), enhances suppressor cell activity (Noronha et al., 1990), reduces IFN-γ production and MHC-II expression (Ling et al., 1985; Panitch, 1992) and, possibly, increases IL-10 production (Porrini et al., 1995). In two large double-blind studies performed in the USA in patients with relapsing–remitting MS, IFN-β was found to inhibit the activity of the disease.

In the first trial, recombinant IFN-β-1b ('Betaseron', Schering) given s.c. every other day was reported to reduce the relapse rate by 31% (0.84/year in the treated group vs 1.27/year in the placebo group), especially at the high dose of 8 MIU, but without significantly affecting the disability status (The IFNB Multiple Sclerosis Study Group, 1993). The number of patients exacerbation-free during this time period was also higher in the high dose protocol (8 MIU×3/week), compared with the placebo recipients. A number of secondary outcome measures were also improved in patients receiving the high dose regimen, including a lower rate of moderate or severe exacerbations and fewer hospitalizations. An important finding in this study was the significant reduction in the MRI T2 burden of disease and a 75–80% reduction in active scans (those showing new or enlarging lesions), in patients administered the high dose (Paty et al., 1993). This effect was long-lasting even after 3–5 years of treatment (The IFNb Study Group, 1995). This treatment was generally well tolerated, but 'flu-like' symptoms (fever, chills, myalgias and fatigue) occurred in the majority of patients (76%), which tended to decrease after the first months of treatment. Injection site irritation was also very common (in 85% of patients), sometimes even severe, persisting for weeks after injection. About a third of the patients developed neutralizing antibodies against 'Betaseron', and this may be a limiting factor in long-term treatment.

In a second study, recombinant glycosylated IFNβ-1a ('Anovex', Biogen) at a dose of 6 MIU im once per week induced a reduction in relapse rate from 0.90/year (placebo) to 0.61/year. The treatment also resulted in a decrease in the number and volume of gadolinium (DTPA)-enhancing lesions in the MRI and, most importantly, delayed the sustained progression of disability and reduced the one and two year progression rates by about 40% (Jacobs et al., 1996). Both 'Betaseron' and 'Avonex' have received the US Food and Drug Administration (FDA) approval for the treatment of ambulatory relapsing–remitting MS.

Another type of recombinant IFN-β ('Rebif', Serono) almost identical to IFN-b la, was recently made available. Although results from large phase III trials are yet to be published, Rebif has shown promising effects (a reduction in the number of relapses and inhibition of MRI activity)

in open phase II studies in Europe (Fieschi et al., 1995; Pozzilli et al., 1996). Preliminary results from the recently completed phase III trial showed significant beneficial effects of Rebif, (6 or 12 million units s.c every other day) in all the clinical and MRI parameters tested in relapsing-remitting MS patients.

4. Conclusion

We have made an attempt to classify the levels at which therapeutic immune intervention is feasible. However it should be emphasized that the immune system functions as a complex network and every treatment has pleiotropic effects at various levels. For example, IFN-β acts at the level of antigen presentation (downregulation), at the level of T-cell activation (anti-proliferative effect), at the cell network level (TH-2/TH-1 balance) and at the cytokine level (reduction of IFN-γ, increase of IL-10 and TGF-β). Similarly, oral tolerization interferes with antigen presentation (anergy induction), at the cellular level (TH-2 shift, increase of suppressor cells) and at the cytokine level (induction of TGF-β). Finally, the immunomodulator linomide seems to affect both antigen presentation (macrophage level) and to induce a cellular shift towards NK-, suppressor- and CD45RA cells. Several immunopathogenetic aspects of MS remain to be clarified. However, recent advances in immunotherapeutic techniques hold out the promise for new horizons in the management of MS with non-toxic, non-immunosuppressive drugs.

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Exhibit B

Targeting of IgMκ Antibodies to Oligodendrocytes Promotes CNS Remyelination

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We previously identified the remyelinating activity of a natural IgMκ oligodendrocyte-reactive autoantibody (SCH94.03), using a virus-induced murine model of multiple sclerosis. We now describe a second mouse IgMκ monoclonal antibody (mAb) (SCH79.08) raised against normal mouse spinal cord homogenate, which reacts with myelin basic protein and also promotes remyelination. Because these two mAbs recognize different oligodendrocyte antigens, several previously identified oligodendrocyte-reactive IgMκ mAbs (O1, O4, A2B5, and HNK-1), each with distinct antigen specificities, were evaluated and found to promote remyelination. In contrast, IgMκ mAbs that did not bind to oligodendrocytes showed no remyelination. One of these, CH12 IgMκ mAb, which shares variable region cDNA sequences with SCH94.03 except for amino acid differences in the complementarity-determining region 3 in both heavy and light chains, did not bind to oligodendrocytes and did not promote remyelination. The fact that multiple oligodendrocyte-reactive antibodies with distinct antigen reactivities induce remyelination argues against direct activation by a unique cell surface receptor. These findings are most consistent with the hypothesis that the binding of mAbs to oligodendrocytes in the lesions induces myelin repair via indirect immune effector mechanisms initiated by the μ-chain. Importantly, these studies indicate that oligodendrocyte-reactive natural autoantibodies may provide a powerful and novel therapeutic means to induce remyelination in multiple sclerosis patients.

Key words: Theiler's virus; oligodendrocytes; demyelination; natural autoantibody; remyelination; multiple sclerosis; immunoglobulin

Demyelination in association with inflammation is the primary structural abnormality in multiple sclerosis (MS). Spontaneous remyelination is limited in the CNS, in part because oligodendrocytes are considered to be postmitotic cells. However, spontaneous remyelination is observed at the edge of MS plaques (Prineas and Connell, 1979). Studies have shown that oligodendrocyte/type-2 astrocyte (O-2A) progenitor cells persist in the adult CNS and proliferate (Wolswijk and Noble, 1989; Armstrong et al., 1992). Alternatively, it has been shown that mature oligodendrocytes can be induced to generate new myelin under the influence of neurons (Wood and Bunge, 1991). Basic fibroblast growth factor induces mature oligodendrocytes to re-enter the cell cycle (Fressinaud et al., 1993: Grinspan et al., 1993) and converts the cells to a novel phenotype (Bansal and Pfeiffer, 1997), providing another cellular mechanism for remyelination.

One of the major goals for the treatment of MS is to develop strategies to promote remyelination. One strategy shown *in vivo* to enhance endogenous myelination has been the use of natural germline antibodies that react to CNS antigens (Miller et al., 1994). This approach is particularly attractive because it rapidly can be translated from the bench to the bedside as a therapy for human MS (Noseworthy et al., 1994; Fazekas et al., 1997).

We demonstrated that a mouse monoclonal antibody (mAb) raised against normal mouse spinal cord homogenate (SCH), designated SCH94.03, enhanced CNS remyelination in the Theiler's murine encephalomyelitis virus (TMEV) model of MS (Miller et al., 1994). SCH94.03 belongs to the IgMκ subclass, is highly polyreactive against known and unknown protein antigens including cytoskeletal proteins, and is encoded by unmutated Ig germline genes, confirming that SCH94.03 is a natural autoantibody (Miller and Rodriguez, 1995a; Asakura et al., 1996a). Of unique importance, SCH94.03 recognizes an unidentified surface antigen on oligodendrocytes (Asakura et al., 1996b), providing a potential target for the mechanism of action of this antibody.

Two major hypotheses have been proposed by which SCH94.03 promotes remyelination. (1) The mAb may bind to a unique receptor on the surface of oligodendrocytes to induce myelination. This hypothesis would predict that only a limited repertoire of Abs with unique specificity would function for myelin repair. (2) The mAb may work by binding to damaged oligodendrocytes and/or myelin, which triggers a cascade of events by other resident CNS cells (i.e., astrocytes, microglia, or neurons) and in turn enhances myelin repair. An attractive hypothesis is that binding to damaged oligodendrocytes and myelin may enhance the opsonization and clearing of CNS debris by macrophages, thus allowing for the normal process of remyelination to ensue. This hypothesis would predict that many polyreactive autoantibodies with specificity to oligodendrocytes and/or myelin would be effective. To address these hypotheses and the mechanism for Ab-mediated CNS remyelination, we set out to identify additional mAbs that promote CNS remyelination in TMEV model and compared their specificities with SCH94.03. In addition, we tested the remyelination-promoting activity of well recognized oligodendrocyte-reactive mAbs O1, O4, A2B5, and HNK-1,

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which previously were shown to have genotypic or phenotypic features of natural autoantibodies (Asakura et al., 1995).

MATERIALS AND METHODS

mAb production and screening. Hybridomas were generated and screened as described (Miller et al., 1994). SJL/J mice were immunized with normal mouse SCH in incomplete Freund's adjuvant, and their splenocytes were fused with NS-1 myeloma cell. Hybridoma supernatants that showed high binding to SCH by ELISA were screened further for their ability to promote remyelination in the TMEV model. Therefore, the Abs were screened on the basis of their ability to promote CNS remyelination rather than for a unique antigen specificity. IgMκ Abs were purified from hybridoma culture supernatants by ammonium sulfate precipitation and dialysis against PBS plus low-ionic-strength precipitation or by affinity chromatography, using goat anti-mouse IgM Ab (μ-chain-specific; Jackson ImmunoResearch, West Grove, PA) bound to carbonyldiimidazole-activated cross-linked agarose (Reacti-Gel 6× matrix, Pierce, Rockford, IL).

Hybridomas and mAb preparation. Hybridomas A2B5, HNK-1, and R24 were purchased from American Type Culture Collection (ATCC, Rockville, MD). Hybridomas O1 and O4 were a gift from Dr. S. E. Pfeiffer (University of Connecticut, Farmington, CT). These hybridomas were cultured in RPMI 1640 supplemented with 10% fetal calf serum (HyClone, Logan, UT) and 2×10^{-2} mM β-mercaptoethanol. B-cell lymphoma CH12 (CH12.Lx) was provided by Dr. G. Haughton (University of North Carolina, Chapel Hill, NC). To obtain secreted IgM from CH12 lymphoma, we stimulated CH12.Lx cells with 50 μg/ml of lipopolysaccharide (Sigma, St. Louis, MO). mAbs O1, O4, and HNK-1 were purified from hybridoma culture supernatant by ammonium sulfate precipitation and dialysis against PBS plus low-ionic-strength precipitation. mAbs A2B5 and CH12 were purified by affinity chromatography. R24 was purified by protein A column. The purity of these mAbs was examined by SDS-polyacrylamide gel electrophoresis, and the immunoreactivity of these mAbs was examined by the immunostaining of rat oligodendrocytes. Control hybridoma XMMEN-OE5 producing antibacterial lipopolysaccharide IgMκ Ab was purchased from ATCC. Clarified ascites of control ABPC22 IgMκ mAb were purchased from Sigma. Both IgMκ mAbs were purified by affinity chromatography, using goat anti-mouse IgM Ab. The purity of the mAbs was confirmed by SDS-polyacrylamide gel electrophoresis.

Virus and animals. The Daniel's strain of TMEV was used for these experiments. Female SJL/J mice from the Jackson Laboratories (Bar Harbor, ME) were used after 1 week of rest. Mice from 4 to 6 weeks of age were injected intracerebrally with 2×10^5 plaque-forming units (pfu) of TMEV in a 10 μl volume. The handling of all animals was in accordance with the institutional guidelines prescribed by National Institutes of Health and Mayo Clinic.

mAb treatment and quantitative morphometry of remyelination. Chronically infected mice (5–6 months after infection) were given intraperitoneal injections of mAb twice weekly for 5 weeks (50 μg/injection). The total dose of each Ab was 0.5 mg. The mice were killed 2 weeks after the completion of mAb treatment. Light microscopic sections were prepared as described (Miller et al., 1994). Mice were anesthetized with pentobarbital, exsanguinated by cardiac puncture, and fixed by intracardiac perfusion with Trump's fixative (phosphate-buffered 4% formaldehyde containing 1.5% glutaraldehyde, pH 7.2). The entire spinal cord was removed and sectioned into 1 mm transverse blocks. Every third block was post-fixed in 1% osmium tetroxide and embedded in Araldite (Polysciences, Warrington, PA). One micrometer sections were cut and stained with *p*-phenylenediamine. Two spinal cord sections of each mouse were examined. The total areas of white matter, demyelination, and remyelination on each section were quantitated by a Zeiss (Oberkochen, Germany) interactive digital analysis system. The area of demyelination was characterized by cellular infiltrates, macrophages engulfing myelin debris, and naked axons. Abnormally thin myelin sheaths relative to axon diameter were used as the criterion for remyelination by oligodendrocytes. Those remyelinated fibers were identified readily (as shown in Fig. 1). Remyelinated areas were defined as a cluster of at least 10 remyelinated fibers. Spontaneous remyelination by Schwann cells also was present rarely in the spinal cord lesions (Miller and Rodriguez, 1995b). Remyelination by Schwann cell was characterized by abnormally thick myelin sheaths relative to axon diameter, with a one-to-one relationship between axons and Schwann cells. These peripheral-type remyelinated areas were excluded from the quantitation. The quantitation was done on coded sections without previous knowledge of the treatment to avoid bias. Statistical comparison between groups in the extent of demyelination and remyelination was performed with an unpaired Student's *t* test.

Cell culture and immunocytochemistry. Oligodendrocytes, astrocytes, and microglia were isolated from telencephalon of newborn Sprague Dawley rats as described (Asakura et al., 1996b). Abs were diluted in PBS. Surface staining was performed at 4°C for 15 min on unfixed cells after they were blocked with PBS containing 3% normal goat serum (NGS). Cytoplasmic antigen staining was performed on cells fixed for 10 min at 4°C with 2% paraformaldehyde and treated for 5 min at room temperature with 0.1% Triton X-100 in PBS, followed by blocking with 3% NGS in PBS. After incubation with the primary Abs and the secondary FITC-conjugated anti-mouse IgM (μ-chain-specific) Ab (Jackson ImmunoResearch), the slides were mounted in MOWIOL (Aldrich Chemical, Milwaukee, WI) containing 2.5% 1,4-diazobicyclo-[2.2.2]-octane (DABCO: Sigma) and were viewed with an epifluorescent microscope.

Immunohistochemistry. Fresh-frozen sections (10 μm) were prepared from various organs of neonatal (postnatal days 7 and 14) rats. Fresh-frozen sections were immunostained with primary Abs and then were fixed with 4% paraformaldehyde or were lightly fixed with acetone and then were immunostained with primary Abs. Bound Abs were detected by fluorochrome-conjugated secondary Ab or by the avidin–biotin immunoperoxidase technique, using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA). MOPC104E (Sigma) mouse IgM mAb was used as an isotype control.

Direct ELISA. Protein antigens, including human red blood cell (RBC) spectrin, bovine myosin (heavy chain), mouse albumin, mouse hemoglobin, mouse transferrin, hen egg lysozyme (HEL), rabbit actin, rabbit myelin basic protein (MBP), and keyhole limpet hemocyanin (KLH), were purchased from Sigma. Proteins were tested for purity by SDS-polyacrylamide gel electrophoresis. All chemical haptens were purchased from Sigma and coupled to bovine serum albumin (BSA) (Miller and Rodriguez, 1995a). Protein antigens were used at 5 μg/ml and haptens at 2 μM. The proteins and hapten–BSA antigens were coated onto polystyrene or polyvinyl chloride microtiter plates in 0.1 M carbonate buffer, pH 9.5, for 18 hr at 4°C. Coated plates were blocked with PBS containing 5% nonfat dry milk and 0.05% Tween 20 for 2 hr at room temperature and were incubated with mAbs diluted in blocking buffer for 4 hr at room temperature. TEPC183 (Sigma) and XMMEN-OE5 IgMκ mAbs were used as isotype control Abs. Bound IgM was detected with biotinylated goat anti-mouse IgM (μ-chain-specific; Jackson ImmunoResearch), followed by alkaline phosphatase conjugated to streptavidin, with *p*-nitrophenylphosphate as the chromogenic substrate. Absorbance was determined at 405 nm.

Immunoblotting. Purified TMEV (Njenga et al., 1996) and rabbit MBP (Sigma) were separated by SDS-polyacrylamide gel electrophoresis on 15% acrylamide gels. Proteins were transferred to a nitrocellulose membrane by electroblotting. The membrane was blocked with Tris-buffered salinc containing 5% nonfat dry milk and 0.03% Tween 20 for 2 hr at room temperature. The membrane was incubated with SCH79.08, SCH94.03, O1, O4, A2B5, HNK-1, CH12, R24, control IgM (MOPC104E), rabbit polyclonal anti-TMEV (1:2000; Njenga et al., 1996), and rabbit polyclonal anti-MBP (1:200; Dako, Carpinteria, CA) Abs for 4 hr at room temperature. SCH79.08, SCH94.03, O1, O4, A2B5, HNK-1, CH12, R24, and control IgM were used at the same concentration (10 μg/ml). Bound Abs were detected with biotinylated secondary Abs (Jackson ImmunoResearch) and alkaline phosphatase-conjugated streptavidin, using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (BCIP/NBT).

RESULTS

IgMκ mAb SCH79.08 promotes CNS remyelination

After cell fusion and cloning, a large panel of mAbs (~100) was screened by ELISA. One mAb, designated SCH79.08 and belonging to the IgMκ subclass, showed significant binding to SCH by ELISA. SJL/J mice chronically infected with TMEV and treated with SCH79.08 showed significantly greater CNS remyelination than animals treated with PBS or isotype-matched control mAb (Table 1). On average, ~20% of the total demyclinated area showed CNS remyelination in mice treated with SCH79.08 ($p < 0.05$), as compared with 2–5% in animals treated with PBS or isotype-matched mAb, respectively. Treatment with SCH79.08

Table 1. Enhancement of CNS remyelination by SCH79.08

Treatment	Number of mice	Area of white matter (mm^2)	Area of demyelinated lesion (mm^2)	Area of CNS-type remyelination (mm^2)	Area of CNS-type remyelination/area of lesions (%)
SCH79.08	15	8.42 ± 0.33	1.01 ± 0.16	0.20 ± 0.05	20.2 ± 4.7
ABPC22	7	8.06 ± 0.46	1.05 ± 0.22	0.05 ± 0.01	4.9 ± 1.4
PBS	6	8.89 ± 0.26	1.01 ± 0.21	0.03 ± 0.01	2.4 ± 0.8

Values represent the mean ± SEM. Statistics by Student's t test of the percentage of area of CNS-type remyelination per area of lesions in mice treated with SCH79.08 as compared with mice treated with ABPC22 (isotype control) or PBS revealed $p < 0.05$.

had no effect on the extent of demyelination (Table 1). Remyelinated lesions were characterized by hundreds of axons with thin myelin sheaths and a relative absence of inflammatory cells or macrophages (Fig. 1). In contrast, most lesions in mice treated with the control IgMκ mAb ABPC22 or PBS had few, if any, remyelinated axons, and the lesions contained intense inflammation and macrophage infiltration (Fig. 1).

mAb SCH79.08 is polyreactive and reacts with MBP

To characterize the antigens recognized by SCH79.08, we performed immunochemistry, ELISA, and Western blotting. By immunocytochemical study, SCH79.08 strongly stained the cytoplasmic structure of most of the cultured cells (Fig. 2*D*). The pattern of reactivity resembled the staining with SCH94.03 (Fig. 2*B*), presumably to a cytoskeletal protein. In contrast to the live surface staining of oligodendrocytes, oligodendrocytes at specific stages of differentiation did not react on the surface with SCH79.08 (Fig. 2*C*). Immunohistochemical staining of fresh-frozen rat tissue sections showed that SCH79.08 is reactive to multiple organs, including brain (predominantly white matter glial cell population), small intestine (lamina propria), and kidney (mesangial cells). These results are consistent with the conclusion that SCH79.08 is polyreactive.

To assess further the polyreactivity of SCH79.08, we performed ELISA, using a panel of protein antigens and chemical haptens. SCH79.08 showed prominent reactivity toward RBC spectrin, but it also reacted with rabbit actin, rabbit MBP, KLH, and mouse hemoglobin (Fig. 3*A*). SCH79.08 also showed reactivity toward multiple chemical haptens, including phenyloxazolone (PhoX), (4-hydroxy-3-nitrophenyl)acetyl (NP), and azophenyltrimethyl ammonium (TMA) (Fig. 3*B*). No reactivity was detected with the carrier protein BSA. Control IgM mAb did not react with any of the protein antigens and chemical haptens that were tested (Fig. 3*C*,*D*). By Western blotting, SCH79.08 showed reactivity against the 21.5 and 18.5 kDa isoforms of rabbit MBP, confirming the ELISA result (Fig. 4*B*).

To exclude the possibility that remyelination was the consequence of SCH79.08 reacting with TMEV, we performed Western blotting, using purified TMEV. SCH79.08 did not react with any of the known TMEV capsid proteins (Fig. 4*A*).

Variable region cDNA sequences of SCH79.08 are different from those of the prototypic remyelination-promoting Ab SCH94.03

Despite binding similarities, variable region cDNA sequences of SCH79.08 were completely different from those of SCH94.03 (Miller and Rodriguez, 1995a) (cDNA sequences of SCH79.08 are available from the GenBank database under accession numbers U91317 and U92070). SCH79.08 VH belonged to the VH558 family. The D segment was derived from the germline DQ52 gene. The JH region was identical to the JH2 germline gene. The Vκ segment for the light chain belonged to the Vκ24 family, whereas the Jκ segment was identical to the Jκ5 germline gene.

Multiple oligodendrocyte-reactive IgMκ mAbs with unique antigen specificities promote CNS remyelination

Of interest, SCH94.03 and SCH79.08 have important similarities with a number of well characterized oligodendrocyte-reactive mAbs. Mouse IgMκ mAbs O1, O4 (Sommer and Schachner, 1981), A2B5 (Eisenbarth et al., 1979), and HNK-1 (Abo and Balch, 1981) recognize unique differentiation stage-specific surface antigens on oligodendrocytes. O1 recognizes multiple lipid antigens, including galactocerebroside, monogalactosyl-diglyceride, and psychosine (Bansal et al., 1989); O4 recognizes proligodendroblast antigen and sulfatide (Bansal et al., 1989, 1992); and A2B5 recognizes ganglioside GQ1c and other gangliosides (Kasai and Yu, 1983; Fredman et al., 1984). The carbohydrate epitope on myelin-associated glycoprotein (MAG) appears to be the principal antigen recognized by HNK-1 (McGarry et al., 1983). This carbohydrate epitope recognized by HNK-1 is also present in other cell adhesion molecules in the nervous system. Similar to SCH94.03 and SCH79.08, these mAbs all belong to the IgMκ subclass, are polyreactive, recognize distinct antigens on oligodendrocytes, and recognize intracellular structures of many cell types. In addition, variable region cDNA sequences of these mAbs have indicated minimal mutations from the germline Ig genes, a characteristic feature of natural autoantibodies (Asakura et al., 1995).

On the basis of these striking similarities, we tested the therapeutic efficacy of oligodendrocyte-reactive mAbs O1, O4, A2B5, and HNK-1 in the TMEV model. A mouse IgG3 mAb R24, which recognizes ganglioside GD3 expressed on O-2A progenitor cells, and an irrelevant mouse IgMκ mAb (XMMEN-OE5) without reactivity to oligodendrocytes also were tested to determine whether Ig isotype and specificity to a unique oligodendrocyte differentiation stage were critical for function. SJL/J mice treated with oligodendrocyte-reactive mAbs O1, O4, A2B5, and HNK-1 showed significantly enhanced CNS remyelination as compared with SJL/J mice treated with control IgMκ or PBS (Table 2). Approximately 20–24% of the area of demyelination was remyelinated in mice treated with O1, O4, A2B5, and HNK-1. Remyelinated lesions in mice treated with O1, O4, A2B5, and HNK-1 were characterized by axons with thin myelin sheaths relative to axon diameter and a relative absence of inflammation (see Fig. 1). In contrast, mice treated with R24 did not show significantly enhanced remyelination (Table 2; Fig. 1). Of interest, these mAbs had no effect on the extent of demyelination and were not pathogenic (Table 2).

O1, O4, A2B5, HNK-1, and R24 did not react with any of the

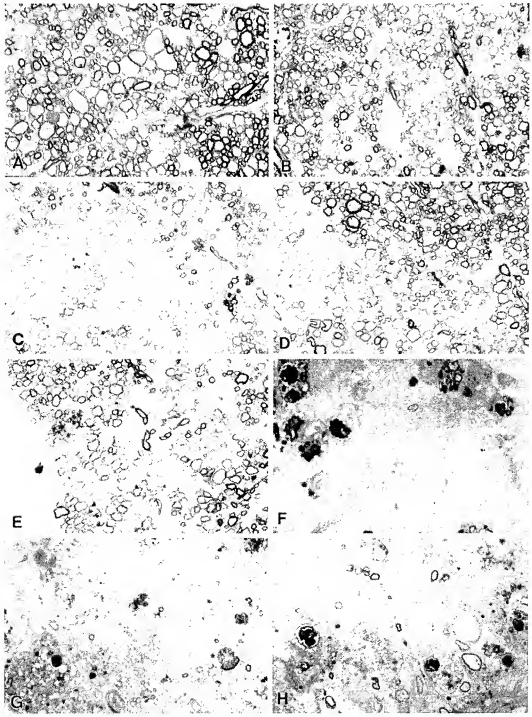

Figure 1. Light micrograph demonstrating extensive CNS remyelination after treatment with SCH79.08, O1, O4, A2B5, and HNK-1. Sections are from the spinal cords of SJL/J mice chronically infected with TMEV. Note the CNS remyelination, characterized by abnormally thin myelin sheath as compared with axon diameter, in demyelinated lesions of mice treated with SCH79.08 (*A*). O1 (*B*), O4 (*C*), A2B5 (*D*), and HNK-1 (*E*). Note the demyelination without significant remyelination in mice treated with R24 (*F*), CH12 (*G*), and control IgMκ ABPC22 (*H*). Araldite-embedded sections were stained with 1% *p*-phenylenediamine (magnification, 875×).

capsid proteins of TMEV, as confirmed by Western blotting (Fig. 4*A*). Immunocytochemical study showed that, although R24 reacted with the surface of O-2A progenitor cells, it did not stain intracellular structures of glial cells (see Fig. 2). By ELISA, R24 did not react with any of the protein antigens and chemical haptens that were examined (data not shown), indicating that R24 is not polyreactive in contrast to O1, O4, A2B5, and HNK-1 (Asakura et al., 1995).

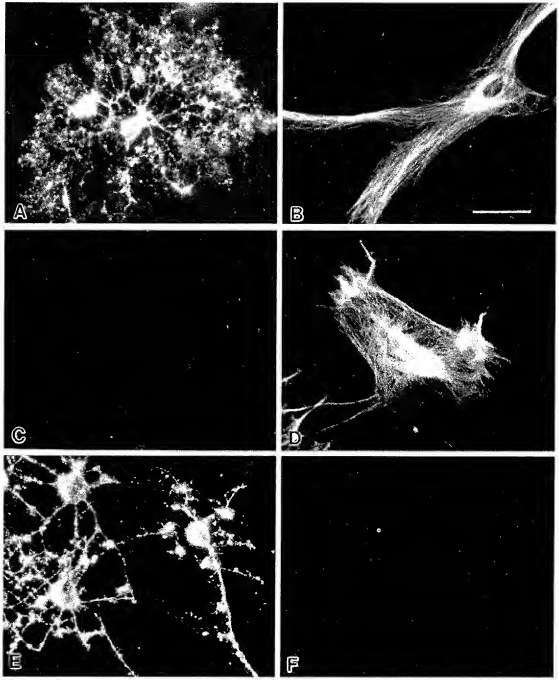

Figure 2. Indirect immunofluorescence of cultured glial cells. Note the live surface staining of oligodendrocytes with SCH94.03 (*A*) and R24 (*E*) but the absence of surface staining with SCH79.08 (*C*). Also note intracellular staining of the cytoplasm of astrocytes with SCH94.03 (*B*) and SCH79.08 (*D*) but the absence of staining with R24 (*F*). Scale bar, 20 μm. Oligodendrocytes and astrocytes were isolated from telencephalon of newborn Sprague Dawley rats.

Complementarity-determining region 3 of Ig is crucial for enhanced remyelination

Having established that a unique family of oligodendrocyte-reactive Abs, each with distinct surface antigen reactivities, would induce remyelination, it was critically important to determine whether the complementarity-determining regions (CDR), which form the Ab-binding site, are essential. Mouse B-cell lymphoma CH12 (CH12.Lx cell) secretes IgMκ mAb under stimulation with lipopolysaccharide. This secreted IgMκ mAb CH12 is encoded by exactly the same germline Ig genes as the genes of SCH94.03; the nucleotide and amino acid differences between SCH94.03 and CH12 exist only in the CDR3 of heavy and light chains (Miller and Rodriguez, 1995a). Despite this structural similarity, CH12 did not stain the surface of oligodendrocytes, did not stain intracellular structure of glial cells (data not shown), and was not polyreactive by ELISA (Miller and Rodriguez, 1995a). TMEV-

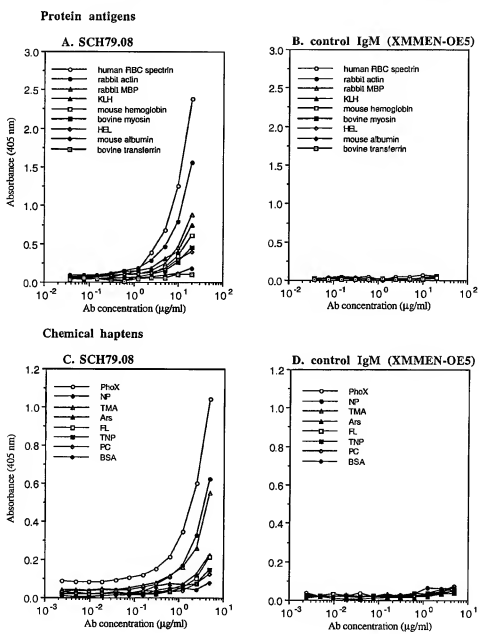

Figure 3. Protein antigen reactivity (*A*) and chemical hapten reactivity (*C*) of SCH79.08 are assessed by direct ELISA. Also shown are protein antigen reactivity (*B*) and chemical hapten reactivity (*D*) of control IgM (XMMEN-OE5). Abbreviations used in these panels: *Ars*, azophenylarsonate; *BSA*, bovine serum albumin; *FL*, fluorescein; *HEL*, hen egg lysozyme; *KLH*, keyhole limpet hemocyanin; *MBP*, myelin basic protein; *NP*, (4-hydroxy-3-nitrophenyl)acetyl; *PC*, azophenylphosphoryl-choline; *PhoX*, phenyloxazolone; *RBC*, red blood cells; *TMA*, azophenyltrimethyl ammonium; *TNP*, trinytrophenyl acetyl. No reactivity to these protein antigens or chemical haptens was detected with another control IgM mAb (TEPC 183) (data not shown).

infected SJL/J mice treated with CII12 IgMκ mAb did not show significant CNS remyelination when compared with control groups (Table 2; Fig. 1).

DISCUSSION

In this study we demonstrated that a panel of oligodendrocyte-reactive IgMκ mAbs promotes remyelination in a virus model of MS. An IgMκ mAb SCH79.08, isolated from mice immunized with emulsions of homogenized normal mouse spinal cord, is polyreactive, binds to MBP, and enhances myelin repair. In addition, other well recognized oligodendrocyte-reactive IgMκ mAbs (O1, O4, A2B5, and HNK-1) also promote CNS remyelination. The results support the hypothesis that the targeting of polyreactive Abs to oligodendrocytes has the potential to promote CNS remyelination.

Although cDNA sequence analysis revealed that variable regions of SCH79.08 are completely different from those of the prototypic remyelination-promoting mAb SCH94.03 in both

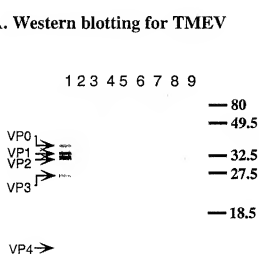

Figure 4. Western blotting of TMEV proteins (*A*) and MBP (*B*). Proteins from purified TMEV and rabbit MBP (obtained from Sigma) were separated on 15% SDS polyacrylamide gels. Bound Ig was detected with alkaline phosphatase-conjugated secondary antibodies by using BCIP/NBT. Molecular weight markers are indicated in kDa at the *right* or *left margin*. *A*, *Lane 1*, Polyclonal rabbit anti-TMEV Ab; *lane 2*, SCH79.08; *lane 3*, O1; *lane 4*, O4; *lane 5*, A2B5; *lane 6*, HNK-1; *lane 7*, CH12; *lane 8*, R24; *lane 9*, control mouse IgM (MOPC 104E). *Arrows* indicate TMEV capsid proteins. *B*, *Lane 1*, Control mouse IgM (MOPC 104E); *lane 2*, SCH94.03; *lane 3*, polyclonal rabbit anti-MBP Ab (obtained from Dako); *lane 4*, SCH79.08. *Arrows* indicate two MBP isoforms (21.5 and 18.5 kDa) recognized by SCH79.08.

heavy and light chains and that these mAbs were selected exclusively for their ability to promote remyelination and not on the basis of antigen specificity, this characterization showed that SCH79.08 has remarkable similarities with SCH94.03. Both mAbs (1) belong to the IgMκ subclass (Miller et al., 1994); (2) are multi-organ reactive (Miller et al., 1996) and polyreactive toward multiple protein antigens and chemical haptens by ELISA (Miller and Rodriguez, 1995a); (3) strongly stain cytoplasmic structures of most cells by immunofluorescence in a similar staining pattern of cytoskeletal proteins (Asakura et al., 1996b); and (4) recognize oligodendrocyte-specific autoantigens: SCH79.08 reacting with MBP and SCH94.03 binding to an uncharacterized surface antigen on oligodendrocytes (Asakura et al., 1996b). These results indicate that SCH79.08 has the characteristic features of a natural autoantibody, which is produced by autoreactive B-cells and is known to exist in healthy humans and rodents (Avrameas and Ternynck, 1993). The fact that both SCH79.08 and SCH94.03 bind to oligodendrocytes, but react to different antigens on these cells, supports the indirect hypothesis of Ab-mediated CNS remyelination.

Besides SCH79.08 and SCH94.03, other oligodendrocyte-reactive IgMκ mAbs O1, O4, A2B5, and HNK-1 also promote CNS remyelination in the TMEV model of MS. Despite the fact that all of the mAbs that promote CNS remyelination are IgMκ, there is no obvious common pattern in germline Ig gene usage of these mAbs (Asakura et al., 1995). Although R24 binds to oligodendrocyte progenitors, it does not promote remyelination possibly because of its IgG isotype, lack of polyreactivity by immunocytochemistry and ELISA, or reactivity to oligodendrocytes of an earlier developmental stage. CH12, which has been classified as a natural autoantibody (Dighiero et al., 1987), was not widely polyreactive (Miller and Rodriguez, 1995a), did not bind to oligodendrocytes, and did not promote remyelination. Taken in concert, this indicates that only a unique population of polyreactive natural autoantibodies with oligodendrocyte reactivity, irrespective of antigen specificity, is effective for treatment. To this point only Igs of the μ-isotypes have induced remyelination, suggesting the possibility that effector functions preferentially mediated by the μ-heavy chain are central to this process. Additionally, the biological difference between SCH94.03 and CH12 establishes that the CDR3 of Ig is critical for the remyelination-promoting activity for this group of Abs and supports the hypothesis that the ability of these Abs to bind within the demyelinated lesions is important to the induction of remyelination.

The mAbs that promote remyelination recognize different differentiation stages of oligodendrocytes from progenitor to mature, suggesting that remyelination-promoting activity may be independent of the developmental stage. The direct binding to surviving oligodendrocytes in the lesion could promote their dedifferentiation. Alternatively, the Abs could block the differentiation of oligodendrocytes to sustain their reactivity with growth factors. There is support from *in vitro* studies for these possibilities. MAb O4 was reported to stimulate differentiation of oligodendrocytes (Bansal et al., 1988). Abs to galactocerebroside cause transmembrane signaling in oligodendrocytes (Dyer and Benjamins, 1990). A mAb (R-mAb) that recognizes galactocerebroside, sulfatide, and a developmentally regulated unidentified antigen on oligodendrocytes reversibly blocks oligodendrocyte progenitor cell differentiation at the late progenitor stage (Bansal and Pfeiffer, 1989). In the presence of R-mAb, mature oligodendrocytes expressing terminally differentiated markers showed a retraction of processes, the formation of swollen cells, and a

Table 2. *In vivo* effects of oligodendrocyte-reactive mAbs and CH12 IgMκ mAb

Treatment	Number of mice	Area of white matter (mm^2)	Area of demyelinated lesion (mm^2)	Area of CNS-type remyelination (mm^2)	Area of CNS-type remyelination/area of lesions (%)
O1	6	7.57 ± 0.52	0.53 ± 0.10	0.14 ± 0.04	24.8 ± 6.2*
O4	7	8.01 ± 0.16	0.84 ± 0.10	0.17 ± 0.05	20.4 ± 4.2*
A2B5	7	7.28 ± 0.38	0.70 ± 0.18	0.18 ± 0.05	24.6 ± 4.6*
HNK-1	7	7.16 ± 0.38	0.78 ± 0.10	0.15 ± 0.03	20.6 ± 2.8**
CH12	7	7.43 ± 0.28	0.57 ± 0.11	0.05 ± 0.02	8.5 ± 4.6
R24	7	7.52 ± 0.34	0.65 ± 0.13	0.04 ± 0.01	6.7 ± 2.5
XMMEN-OE5	6	9.32 ± 0.42	1.06 ± 0.27	0.03 ± 0.01	3.4 ± 1.0
PBS	6	7.46 ± 0.76	0.51 ± 0.15	0.05 ± 0.02	8.0 ± 2.2

Values represent the mean ± SEM. Statistics by Student's t test of the percentage of area of CNS-type remyelination per area of lesions in mice treated with oligodendrocyte-reactive mAbs (O1, O4, A2B5, and HNK-1) as compared with mice treated with XMMEN-OE5 (control IgM) or PBS revealed *$p < 0.05$; **$p < 0.01$. R24 is an IgG3 mAb, which recognizes ganglioside GD3 expressed on O-2A progenitor cells. CH12 is encoded by the same germline Ig genes of SCF94.03, and the amino acid differences between them exist only in the CDR3.

reduction of the levels of terminally differentiated markers (Bansal and Pfeiffer, 1994). MAG, which is recognized by HNK-1, has been shown to be a major inhibitor of axonal regeneration *in vitro* (McKerracher et al., 1994; Mukhopadhyay et al., 1994), although its inhibitory activity of axonal regeneration *in vivo* remains inconclusive (Bartsch et al., 1995; Schäfer et al., 1996). Possibly, HNK-1 may promote CNS remyelination by interfering with MAG expression.

The observation that multiple oligodendrocyte-reactive Abs, each with distinct antigen specificities, promote remyelination is most consistent with the hypothesis that direct binding of the mAbs to injured oligodendrocytes in the lesion induces myelin repair via an immune effector mechanism initiated by the μ-heavy chain. Consistent with this hypothesis, we previously reported that affinity-purified polyclonal anti-MBP Abs promote CNS reinvelination; therefore, reactivity to an intracellular marker of mature oligodendrocytes is also effective for myelin repair (Rodriguez et al., 1996). In further support of the hypothesis, all mAbs that promoted remyelination not only bound to oligodendrocytes but also reacted with intracellular antigens. One possibility is that IgM binding to damaged cells enhances their removal by scavenger macrophages and microglia so that healthy oligodendrocytes or O-2A progenitor cells can initiate their myelination program.

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